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Critical biological processes such as energy generation and signal transduction are driven by the flow of electrons and ions across the membranes of living cells. As a result, there is substantial interest in creating nanostructured materials that control transport of these charged species across biomembranes. This review describes recent advances in the synthesis of *de novo* and protein nanostructures for transmembrane ion and electron transport and the mechanistic understanding underlying this transport. This body of work highlights the promise such nanostructures hold for directing transmembrane transport of charged species as well as challenges that must be overcome to realize that potential.

1. Introduction

Across all domains of life, cellular membranes form the active barrier separating the molecules and conditions for life from the non-living environment, thus literally dividing life from death. Archaea, gram-positive bacteria, and eukaryotes use a single membrane to separate their internal contents from their environment, while gram-negative bacteria use a set of two membranes spaced ca.10-50 nm apart to divide internal and external spaces. The core structural component of these membranes is the lipid bilayer. The lipid bilayer is a 2D fluid, composed of two opposing leaflets of lipids, each of which has a hydrophilic head group ca.1 nm in length and two hydrophobic tail groups which span 2-3 nm range. The lipid bilayer is highly impermeable to most ions and most polar molecules, including redox carriers, and thus acts primarily (although not exclusively) as a permeability barrier.

Membrane proteins, the other major type of molecules in the membrane, confer much of the function to the membrane. Many of these proteins enable selective active and passive transport of ions, polar molecules, and water across the membrane. The hierarchy of membrane transporters is rich and diverse: porins transport larger ions and molecules, ion channels form defined pores permeable only to specific ions, ionophores and transporters shuttle specific ions and/or molecules across the membrane, and pumps drive ions across the membrane against the concentration gradient. A second prominent class of membrane proteins are electron transfer proteins, which carry electrons, originally generated from oxidization of organic or inorganic electron donors, from donors to acceptors on the other side of the lipid bilayer. These proteins utilize a variety of redox centers, including flavins, hemes, iron-sulfur clusters and even cysteines, to operate across a ~1 V range of redox potentials. Electron transfer proteins also contain molecular recognition elements that enable exquisitely selective transport from specific electron donors to specific acceptors.

The highly-regulated transport of both ions and electrons by membrane proteins underpins a number of central biological processes: respiration, photosynthesis, signal transduction, and transport of nutrients and waste products. The movement of electrons across cellular membranes is frequently coupled to proton translocation, thus creating a proton-motive force across the membrane. Likewise, regulation of ion flow across lipid bilayers directly regulates the electrochemical gradient of that ion and the overall membrane potential. These electrochemical gradients power ATP synthesis, selective transport of ions and molecules, cellular motility, heat production, and redox balance. Hence, modulating transmembrane flux of ions or electrons may allow monitoring and control of a wide-range of intracellular biological processes, such as molecular sensing, signal transduction, biosynthesis, energy generation, cell growth, and cellular movement. In short, nanostructures that regulate ion and electron flow have the capacity to form synthetic bio-electronic interfaces that seamlessly exchange information and energy across the boundary between living and human-made systems.

While researchers have come up with a large number of *de novo* designed nanostructures that mimic many of the membrane transport functions, it is fair to say that the complexity of these structures still have not caught up with the diversity and refinement seen in membrane proteins. Nonetheless, the simplicity of some of these artificial transporters gives researchers an opportunity to study the fundamental physical principles of membrane ion and electron transport. In a complementary approach, researchers have recently begun re-engineering Nature's nanostructures, membrane proteins, to control charge transfer in new cellular contexts, e.g. in different cells types or under specific environmental conditions. While the synthesis of these non-canonical nanostructures poses a significant technical challenge, these structures offer highly selective recognition of redox partners which cannot be matched by designed nanostructures. In this review, we describe recent developments in

the use of *de novo* and biologically-derived nanostructures to move ions and electrons across biomembranes and the mechanistic understanding gleaned from these studies.

2. Nanostructures that move electrons across cellular membranes

While cells (usually microorganisms) that can transfer electrons between intracellular and extracellular species do exist, they are not prevalent, nor are they widely used in biotechnology. Thus widening the variety of cell types in which electrons can be directed across membranes is of general interest and is a ripe opportunity for interdisciplinary nanoscience. Broadly speaking, to transport electrons across the membrane, membrane-spanning nanostructures must have either a delocalized electron system (**Figure 1a**) , i.e. a conduction band or conjugated π system, or a set of discrete redox active groups within hopping distance (~1-2 nm) (Figure 1b) that span the 5 nm-thick membrane. In the next section, we describe recent advances utilizing both of these strategies to accelerate electron transport across cellular membranes.

2.1 Inorganic and oligomeric nanostructures for transmembrane electron transfer

The high conductivity offered by metallic and semiconducting inorganic nanostructures has attracted researchers interested in augmenting transmembrane electron transfer, and early studies suggest that it may be a promising approach. In one example, Zhao and co-workers introduced Pd^{2+} [1] to *Desulfovibrio desulfuricans,* a bacterium capable of metal reduction, to form Pd nanoparticles on the surface. The presence of these nanoparticles boosted the flux of metabolically-derived electrons from the microbes to an external electrode. In a second notable example, electrode bound carbon nanotube (CNT) arrays have been used to enhance current flow out of the microbe *Proteus vulgaris*[2] and to reduce and oxidize a non-cellular redox moiety, methylene blue, entrapped inside mouse macrophage cells.[3] These studies suggest that, with the appropriate functionalization, CNT arrays may be a means of gaining redox access to the interior of multiple cell types. Nonetheless, there still

remains much to be learned about both of these promising systems, most critically an understanding of how these very different nanostructures associate with cellular membranes and the mechanism(s) underlying the increased electron flux from cells to external electrodes.

Lipid-intercalating conjugated oligoelectrolytes, developed by Bazan and co-workers, represent a new class of semiconducting molecules designed to enhance electron transport across cell membranes (Figure 1c). These amphiphiles, such as 4,4′-bis(4′-(N,N-bis(6″-(N,N,N-trimethylammonium)- hexyl)amino)-styryl)stilbene tetraiodide (DSSN+), spontaneously insert into either artificial lipid bilayers or cellular membranes where they become fluorescent.[4] DSSN+ incorporation increases the current across artificial bilayers[4] and from of a variety of microbes including *Saccharoymes cerevisiae*,[4] *Esherichia coli*, *Shewanella oneidensis* MR-1, and even uncharacterized wastewater microbial consortia.[5] However, very recent detailed studies show that different and unexpected mechanisms underlie the current increases in living cells. The increase in current from *E. coli* is due to inadvertent release of redox-active molecules by cell lysis.[6] In contrast, in *S. oneidensis* DSSN+ increases electrode attachment by the microbes and appears to enhance interfacial redox processes between the extracellular surface of the cell and the electrode.[7] Thus, despite the ability of DSSN+ to enhance transmembrane electron transfer in artificial lipid bilayers, currently there is no evidence that DSSN+ increases transmembrane electron transport in living systems on a per cell basis. Nonetheless, the mechanistic investigations of DSSN+ set a laudable precedent that, as it is adopted by more researchers, will strengthen the field as a whole.

Taken together, this recent body of work highlights key advantages and challenges of using conductive and semiconducting nanostructures to facilitate electron transport across cellular membranes. On the upside, the ability to synthesize a nanostructure and test its function in both artificial membranes and multiple organisms has fueled the relatively rapid discovery of different classes of materials, e.g. CNT arrays and oligoelectrolytes, that yield

higher current from multiple cell types. The surprising corollary is that the enhanced current in these different cell types can proceed from mechanisms other than the intended one, and indeed different mechanisms than those in artificial membranes. This divergence probably arises because of structural variations across artificial and cellular membranes from different organisms. These observations underscore the need to pinpoint the specific mechanisms that lead to enhanced current among the many possibilities, e.g. increased membrane permeability, enhanced electrode attachment, faster cell surface/electrode interfacial electron transfer, or increased transmembrane electron flux. Ultimately, the reward of such studies will be the robust design rules for materials that increase electron transport across specific biomembranes.

2.2 Protein nanostructures for transmembrane electron transfer

A newly emergent paradigm is to use electron transfer membrane proteins or protein complexes in non-native contexts as nanostructures (Figure 1b). This unorthodox approach uses synthetic biology to express the genes that code for redox-active membrane spanning proteins or protein complexes at increased levels or in non-native organisms.

This approach was first attempted by Gorton and co-workers to avoid use of exogeneous mediators when using redox-active polymers to 'wire up' cells to extracellular electrodes.[8] When these researchers introduced the electron transfer protein cytochrome b_{558} from *Bacillus subtilis*, which transports electrons as part of NADPH oxidase, into *E. coli* along with the osmium redox polymer, they observed increased current flow out of *E.coli* to an extracellular electrode.[9] This result suggested that electron transfer proteins could be used as biological nanostructures in non-native organisms to increase transmembrane flux.

Building on this approach, several research groups have begun using the proteins of the **m**e**t**al-**r**educing (Mtr) pathway, native to the metal-reducing bacterium *Shewanella oneidensis* MR-1, to increase electron flow across cellular membranes under non-native

conditions. In contrast to the protein used by Gorton and co-workers, the proteins of this pathway have evolved to provide an electron path across the inner and outer membrane to extracellular electron acceptors. CymA, an inner membrane tetraheme cytochrome *c*, moves reducing equivalents from quinols in the inner membrane to the periplasmic face of the inner membrane, and the MtrCAB complex moves reducing equivalents across the outer membrane via a proposed 'porin-cytochrome' structure (Figure 1d).[10] In this proposed structure, MtrA sits inside the MtrB porin and MtrC associates with MtrAB near the membrane surface, leaving most of MtrC exposed to extracellular solution.[11] The Mtr complex can spontaneously insert in the lipid bilayer of vesicles *in vitro* and provide a route for electrons to transverse the membrane.[12] Interestingly, a set of paralogous proteins in *S. oneidensis*, MtrFED, is proposed to form a complex like MtrCAB.[13]

Two pioneering studies have used triggered synthesis of the Mtr complex by its naïve organism to open electron flow out of cells in response to specific environmental conditions. These studies use mutant strains of *S. oneidensis* MR-1 which lack elements of the MtrCAB complex and its paralogs, and thus cannot reduce electrodes. Gescher and co-workers[14] re-designed this mutant strain of *S. oneidensis* MR-1 to express the MtrC homolog, MtrF, in response to arabinose. Thus, upon exposure to arabinose, the resulting MtrFAB electron nanoconduit re-established the ability of the cells to reduce an anode. In a similar vein, Webster *et al*.[15] engineered the biosynthesis of MtrB to be stimulated in the mutant *S. oneidensis* strain in response to arsenic. The resulting system serves as whole cell electronic biosensor of arsenic.

These studies suggested that if inserted in the membrane of a different organism, the Mtr would serve as an efficient electron nanoconduit that could move electrons across that lipid bilayer. Ajo-Franklin and co-workers first successfully demonstrated this approach in the model organism *E. coli.*[16] First, *E. coli* was genetically modified to synthesize the Mtr electron nanoconduit and position it in the outer membrane. These engineered *E. coli* cells

(*mtr* v1) reduced extracellular iron(III)oxide many times faster than *E. coli* cells lacking the Mtr complex[16] (**Figure 2** a,b), albeit many times slower than *S. oneidensis*. To further improve the electron flux, the biosynthesis of Mtr complexes was tuned to vary the number of electron nanoconduits per cell.[17] Intriguingly, an *E. coli* strain that synthesized equivalent levels of Mtr electron nanoconduit (*mtr* v2) as the original strain (*mtr* v1), but did so more efficiently, showed significantly improved electron transfer to an extracellular electrode (Figure 2 c,d).[17] Nonetheless the current level produced by this strain was still quite low, probably because the introduced electron path only bridged one of the two membranes of *E. coli*. To extend the electron transfer path across both membranes, the inner membrane protein CymA was co-expressed with the Mtr complex in *E. coli* (*cymA-mtr* strain). In the presence of an electron donor, the *cymA-mtr E. coli* sustainably generate ~30 fA/cell for weeks,[18] which is a respectable 15-30% of the ~100-200 fA/cell produced by *S. oneidensis*. Relative to control *E. coli*, these cells do not attach to the electrode in greater numbers, nor do they consume electron donor more rapidly. Taken together, these data are most consistent with the increased current production being due to electron flux through the Mtr complex. Most excitingly, the increased current production shifts the metabolic products excreted by *E. coli*, suggesting that the electronic connection afforded by the Mtr electron nanoconduit alters the intracellular redox state.[18] This capability in *E. coli*, the workhorse of industrial biotechnology, opens the door to real-time electronic modulation of cellular metabolism, which has the potential to revolutionize bio-based chemical synthesis.

These studies show that re-engineered membrane proteins offer complementary advantages and disadvantages to inorganic nanostructures as materials to increase electron transport across membranes. Despite major advances in the last five years, programming the biosynthesis of redox-active protein nanostructures remains a significant and organism-specific undertaking. On the other hand, all evidence to date suggest that these electron nanoconduits function in engineered contexts as in their native environment, removing much

of the mechanistic uncertainty associated with *de novo* nanostructures. Moreover, the modest electron flux offered by these protein nanostructures is still sufficiently high to enable biotechnological applications such as whole-cell bioelectronic sensors and electronically-controlled biosynthesis. Nonetheless, this approach must be extended to microorganisms other than *S. oneidensis* and *E. coli* to show that this strategy is truly a general one.

3. Nanomaterials that move ions across cellular membranes

The main structural requirement for a synthetic ion channel is to create a membrane-spanning structure that encloses significant space that is sufficient for passage of certain types of hydrated ions. Thus, a number of *de novo* designed synthetic ion channel structures focused on assembly of distinct subunits into a membrane nanopore. Broadly, the examples of synthetic channels reported in literature can be divided into two classes (**Figure 3, a,b**). Tubular channels, have structures that are akin to the gramicidin channel, in which the transmembrane passage is formed by stacking of several well-defined tubular subunits (Figure 3b). In the second class, aggregate channels are formed by assembly of several membrane-spanning subunits; this mechanism is similar to the formation of the amphotericin channel (Figure 3a) and mimics the common biological pore formation mechanism by membrane oligomerization. The first class of structures include antimicrobial agents tris-macrocycle hydraphiles that use three large crown ether subunits to form a transmembrane structure.[19] Another example of a gramicidin-like structure is a ~2.5 nm diameter rigid-rod β-barrel formed by octiphenyl derivatives.[20] Another popular approach for creating synthetic ion channels focuses on cyclic peptides that form a stacked barrel structure (a so-called peptide nanotube) in the membrane.[21] Barboui and collegues also created water channels using directed stacking of G-quadruplex structures.[22] Aggregate channels typically exhibit less structural precision than the stacked structures, but since they use building blocks that span the membrane (typically two-headed amphiphiles) they can, in principle, give rise to

asymmetric structures that could exhibit voltage-gated functionality. Examples of such channels are based on poly-macrocycles or acyclic oligoesters, and others draw upon same octiphenyl scaffold used in gramicidin-like structures.[21] Despite the significant success of these bottom-up synthetic approaches, these channels remain complex and their transport properties do not always emphasize ion transport efficiency.

A different paradigm aims to reproduce the biological approach to pore channel formation by using a heteropolymeric scaffold that folds into a stable membrane pore structure using directed hydrogen bonding and van der Waals intractions. A striking example of this approach was recently demonstrated by Dietz, Simmel, and co-workers, who used DNA origami scaffold to design a large membrane-spanning ion channel with 2 nm inner diameter, and 6 nm outer diameter, that exhibited ion conductances on par with its biological counterparts.[23] This DNA origami channel also produced "gating" conductance sub-states, and showed the ability to translocate ssDNA and produce characteristic current blockades. DNA-based programmable scaffolds offer perhaps the ultimate flexibility in constructing synthetic membrane ion channels, however use of such constructs in live cells would be complicated by concerns about stability and enzymatic degradation.

Yet another paradigm for membrane ion channel design is based on using inorganic nanotubes, which, in principle, can provide pores with the diameters in the biological nanopore range. Of those pores, CNTs have again attracted the most attention, because their hydrophobic, smooth, and narrow inner pores reproduce a number of key structural features of aquaporin water channels and membrane ion channels. Unsurprisingly, nanotubes are very efficient transporters: MD simulations of water transport predicted very high water flow rates,[24] which were later verified in the experiments that showed large flux enhancements in 3-10 μm long, 1.6 nm diameter carbon nanotubes.[25]

Long individual CNTs are not suitable for incorporation into a cell membrane, and instead are prone to being internalized by the cells via endocytosis; in contrast, we recently

discovered that short carbon nanotubes with lengths comparable to the lipid membrane thickness are capable of self-inserting into the membrane when they are wrapped by the lipid molecules (**Figure 4**).[26] After insertion, they form pore channels in the membrane (Figure 4) that resemble biological porin proteins (hence the term "carbon nanotube porins"). Surprisingly, cryoelectron microscopy data show that the geometry of CNT porins in the membrane is fairly well defined, the nanotube typically sits at a nearly normal angle to the membrane plane (0-15 deg), and one end of the CNT usually abuts the membrane surface, allowing the carboxylic groups on the nanotube to interact with the headgroups of the lipid bilayer.[26]

These carboxylic group at the ends of the CNT porin, together with another main structural element of the CNT porin - the inner cavity of the nanotube - define the porin's transport properties. Planar lipid bilayer measurements (Figure 4) were able to resolve the individual incorporation of CNT porins into the lipid membrane and determined the individual porin conductance (Figure 4). Surprisingly, despite a relatively wide distribution of the porin length, their conductance is much more narrowly defined (Figure 4), which points to the dominant role of the end resistances in this system. In the high ionic strength range of 0.15-2 M, the conductance values measured in the planar lipid bilayer were proportional to the ion concentration in the solution (Figure 4), which indicates that at these ionic strengths the porin is highly permeable to small monovalent ions and that the ions represent the dominant electrophoretic current carrier in these channels.

Bulk scale measurements can explore the selectivity of the CNT porins further.[27] Osmotically-induced vesicle size changes, monitored by dynamic light scattering (Figure 4) provide a convenient means of assessing whether the CNT porin is permeable to a particular osmolyte. In these measurements the osmotic pressure differential shrink the hydrodynamic size of the lipid vesicles only if the CNT porins do not allow the osmolyte species to move across the membrane. The simple structure of the CNT porin argues that the rejection of the

uncharged species should be straightforwardly determined by their size. Indeed, the data comparing the rejection of two sugar molecules of different size showed (Figure 4) that sucrose molecules, which at 0.9 nm are smaller than the 1.5 nm diameter of the CNT porin can pass through the porin, and ca.5 nm dextran molecules are rejected.[27] The ion rejection properties are somewhat more complicated, as they are defined by the electrostatic interactions of an ion with the carboxylate groups at the end of the CNT. This ring of negative charge should repel negatively charged ions, unless the ionic strength is so high that the electrostatic interactions are screened. Indeed, the data show (Figure 4) that the ion rejection characteristics undergo a clear transition going from high ionic strength to low ionic strength where the CNT porins reject ions at low ionic strength values but become permeable to them as the ionic strength increases and the electrostatic field becomes screened. Further analysis of these interactions (Figure, 4 inset) reveals that, despite the peculiar geometry of CNT porins, their rejection behavior is described well by the Donnan model of ion rejection in charged pores.

CNT porins also are able to insert into the membranes of live cells. Remarkably, they readily incorporat into two different mammalian cell lines, chinese hamster ovary (CHO) and human embryonic kidney (HEK-293T). Patch-clamp electrical recordings show that CNT porins insert into the membrane (Figure 4), forming a well-defined pore channels that also exhibit gating behavior similar to native biological channel activity.[26] As expected, individual channel conductance is consistent between the two cell lines, and matches conductance measured for CNT porin incorporation into planar lipid bilayers. These data point to the potential role of the CNT porins as universal synthetic scaffold for forming pore channels in cellular membranes, provided that subsequent research addresses the selectivity concerns.

4. Conclusion

It is an exciting time for the bioelectronic interface community. The one-two punch of advances in synthetic biology and nanomaterial synthesis has delivered an unprecedented degree of control over electron and ion transport in biological systems. Researchers have demonstrated a palette of nanostructures, ranging from carbon nanotube arrays to protein nanostructures, dramatically increase current flow between living cells and external electrode, and these systems are already forming the backbone of next-generation sensing and synthesis applications. *De novo* synthetic membrane pores and nanomaterial-based membrane channels are starting to approach the efficiency of biological channels and novel customizable channel scaffold based on DNA and nanotube frameworks promise to deliver the structural variety of their biological counterparts.

Yet future challenges for these materials await. Whether the electron current increase shown by the current crop of electron transport nanostructures arises through an increased flow of electrons across an intact lipid bilayer remains an unanswered question in many cases. A challenge that looms large for both electron- and ion-transporting nanostructures is effective strategies to introduce selectivity. For electron transporters, there are few means to direct electron flow to or from biomolecules and pathways of our choosing, yet this is necessary to achieve precise control of specific cellular processes. Our understanding of how Nature uses molecular recognition to route electron flow may be critical for non-natural strategies. For ion channel scaffolds we need to develop strategies to discriminate between different types of charged and uncharged species, while still retaining transport efficiency. For example, current ion selectivity of CNT porins at physiological conditions is still far from that of the biological ion channels, and thus more effort is necessary to achieve biologically-relevant selectivity in this artificial channel platform. The strategy for achieving these goals has to be based on deep understanding of the fundamental physics of ion transport in ultra-confined spaces, as well as detailed knowledge of the biological ion transport mechanisms.

These advances would then allow researchers to increase the versatility and utility of *de novo* transmembrane structures and ultimately make the vision of a functional two-directional bioelectronic interface a reality.

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[1] X. Wu, F. Zhao, N. Rahunen, J. R. Varcoe, C. Avignone-Rossa, A. E. Thumser, R. C. T. Slade, Angew. Chem., Int. Ed. 2011, 50, 427.
[2] F. J. Rawson, D. J. Garrett, D. Leech, A. J. Downard, K. H. R. Baronian, Biosens. Bioelectron. 2011, 26, 2383.
[3] F. Rawson, C. Yeung, S. Jackson, P. Mendes, Nano letters 2012, 13, 1.
[4] L. E. Garner, J. Park, S. M. Dyar, A. Chworos, J. J. Sumner, G. C. Bazan, J. Am. Chem. Soc. 2010, 132, 10042.
[5] L. E. Garner, A. W. Thomas, J. J. Sumner, S. P. Harvey, G. C. Bazan, Energy Environ. Sci. 2012, 5, 9449.
[6] V. Wang, N. Yantara, T. M. Koh, S. Kjelleberg, G. C. Bazan, J. Loo, N. Mathews, Chem. Commun. 2014.
[7] N. D. Kirchhofer, X. Chen, E. Marsili, J. J. Sumner, F. W. Dahlquist, G. C. Bazan, Phys. Chem. Chem. Phys. 2014, 16, 20436.
[8] K. Hasan, S. Patil, D. Leech, C. Hägerhäll, L. Gorton, Biochem. Soc. Trans. 2012, 40, 1330.
[9] S. Alferov, V. Coman, T. Gustavsson, A. Reshetilov, C. von Wachenfeldt, C. Hägerhäll, L. Gorton, Electrochim. Acta 2009, 54, 4979.
[10] D. J. Richardson, J. N. Butt, J. K. Fredrickson, J. M. Zachara, L. Shi, M. J. Edwards, G. White, N. Baiden, A. J. Gates, S. J. Marritt, Mol. Microbiol. 2012, 85, 201.
[11] T. A. Clarke, M. J. Edwards, A. J. Gates, A. Hall, G. F. White, J. Bradley, C. L. Reardon, L. Shi, A. S. Beliaev, M. J. Marshall, Z. Wang, N. J. Watmough, J. K. Fredrickson, J. M. Zachara, J. N. Butt, D. J. Richardson, Proc. Natl. Acad. Sci. U. S. A. 2011, 108, 9384.
[12] G. F. White, Z. Shi, L. Shi, Z. Wang, A. C. Dohnalkova, M. J. Marshall, J. K. Fredrickson, J. M. Zachara, J. N. Butt, D. J. Richardson, T. A. Clarke, Proc. Natl. Acad. Sci. U. S. A. 2013, 110, 6346.
[13] D. Coursolle, J. A. Gralnick, Mol. Microbiol. 2010.
[14] F. Golitsch, C. Bücking, J. Gescher, Biosens. Bioelectron. 2013, 47, 285.

[15] D. P. Webster, M. A. TerAvest, D. F. R. Doud, A. Chakravorty, E. C. Holmes, C. M. Radens, S. Sureka, J. A. Gralnick, L. T. Angenent, Biosens. Bioelectron. 2014, 62, 320324.
[16] H. M. Jensen, A. E. Albers, K. R. Malley, Y. Y. Londer, B. E. Cohen, B. A. Helms, P. Weigele, J. T. Groves, C. M. Ajo-Franklin, Proc. Natl. Acad. Sci. U. S. A. 2010, 107, 19213.
[17] C. P. Goldbeck, H. M. Jensen, M. A. TerAvest, N. Beedle, Y. Appling, M. Hepler, G. Cambray, V. Mutalik, L. T. Angenent, C. M. Ajo-Franklin, ACS Synth. Biol. 2013, 2, 150.
[18] M. A. TerAvest, T. J. Zajdel, C. M. Ajo-Franklin, ChemElectroChem 2014, 1, 1874.
[19] C. L. Murray, H. Shabany, G. W. Gokel, Chem. Commun. 2000, 2371.
[20] S. Matile, A. Som, N. Sordé, Tetrahedron 2004, 60, 6405.
[21] T. M. Fyles, Chem. Soc. Rev. 2007, 36, 335.
[22] M. Barboiu, Angew. Chem., Int. Ed. 2012, 51, 11674.
[23] M. Langecker, V. Arnaut, T. G. Martin, J. List, S. Renner, M. Mayer, H. Dietz, F. C. Simmel, Science 2012, 338, 932.
[24] G. Hummer, J. C. Rasaiah, J. P. Noworyta, Nature 2001, 414, 188.
[25] J. K. Holt, H. G. Park, Y. Wang, M. Stadermann, A. B. Artyukhin, C. P. Grigoropoulos, A. Noy, O. Bakajin, Science 2006, 312, 1034.
[26] J. Geng, K. Kim, J. Zhang, A. Escalada, R. Tunuguntla, L. R. Comolli, F. I. Allen, A. V. Shnyrova, K. R. Cho, D. Munoz, Nature 2014, 514, 612.
[27] K. Kim, J. Geng, R. Tunuguntla, L. R. Comolli, C. P. Grigoropoulos, C. M. Ajo-Franklin, A. Noy, Nano letters 2014, 14, 7051.

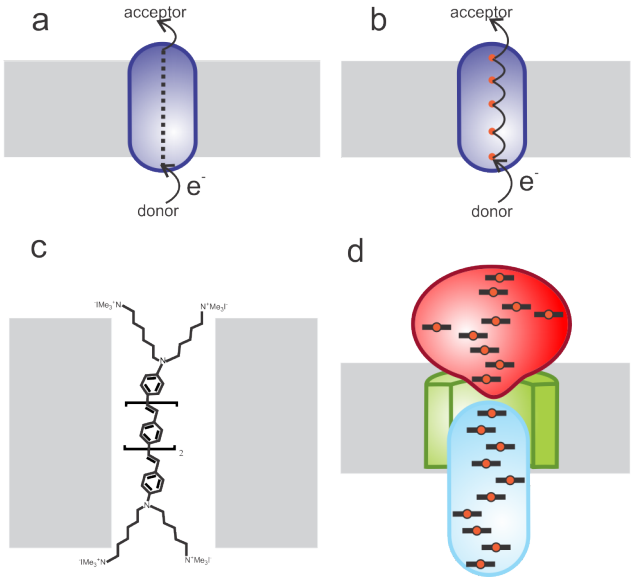

Figure 1. Schematics of membrane-spanning nanostructures designed to transport electrons cross a lipid bilayer. (a) Metallic or semiconducting nanostructures have delocalized electron systems that potentially offer very rapid electron transport across the membrane, while (b) redox-active protein nanostructures afford discrimination between different donors and acceptors through molecular recognition elements. (c) Conjugated oligoelectrolytes are one prominent example of a semiconducting nanostructures which span lipid membranes. (d) The Mtr complex of *S. oneidensis* MR-1 is a noteworthy example of protein nanostructures that enhance electron flux across cellular membranes.

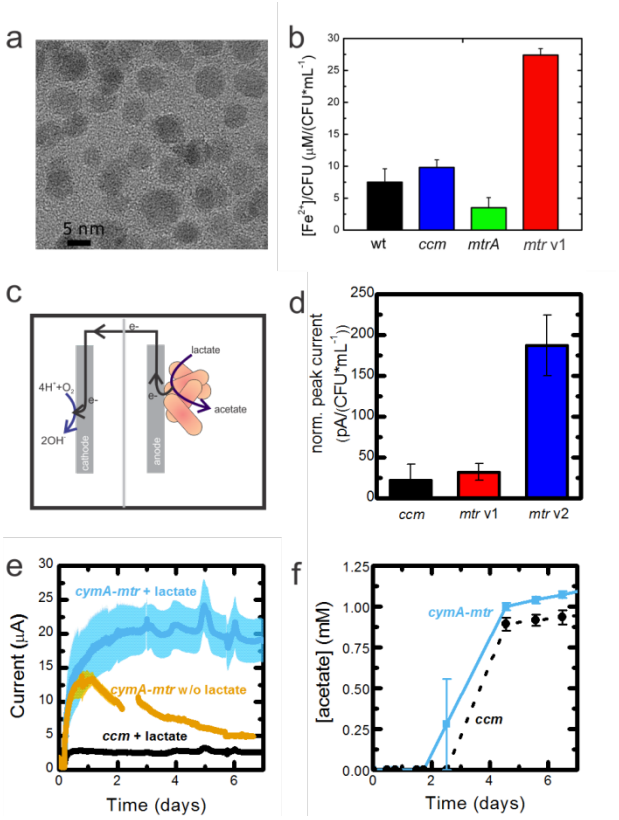

Figure 2. The Mtr electron nanoconduit increases the electron flux across the *E. coli* cell membrane and permits intracellular processes to be modulated by an external electrode. (a) Iron (III) oxide nanoparticles supplied to *E. coli* are (b) reduced to Fe^{2+} at a ~4-fold faster rate by *E. coli* containing the Mtr electron nanoconduit (*mtr* v1, red) than control strains of *E. coli*. (c) Schematic of a microbial electrochemical cell used to test the ability of cells to transfer electrons to an electrode. (d) *E. coli* that efficiently biosynthesize Mtr electron nanoconduits (*mtr* v2, blue) reduce carbon electrodes faster than *E. coli* without Mtr (*ccm*, black) or *E. coli* that inefficiently synthesize Mtr (*mtr* v1, red). (e) *E. coli* containing CymA as well as the Mtr electron nanoconduit (*cymA-mtr*) sustain current production only when an electron donor is present (blue). (f) Engineered *cymA-mtr E. coli* produce more oxidized metabolites, e.g. acetate, than *E. coli* without Mtr (*ccm*, black). The resulting increase in electron flux is consistent with the increase in current production. Panel a,b: Reproduced with permission.[16] Copyright 2010 by the National Academy of Sciences of the United States of America.

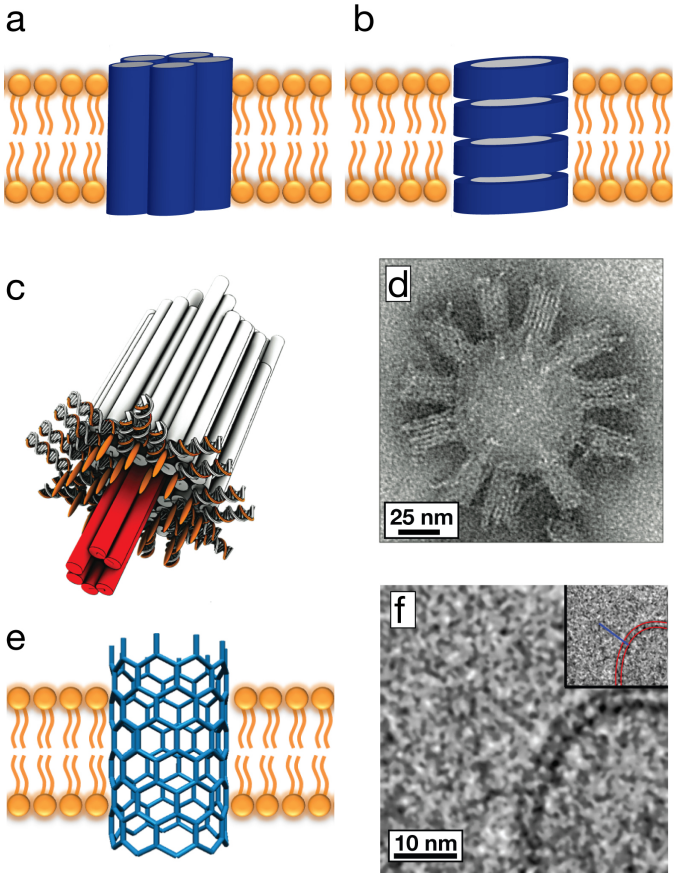

Figure 3. Artificial membrane ion channels. (a,b) Structures of an aggregate (a) and stacked (b) artificial membrane channels. (c) Schematics of a DNA origami-based membrane channel formed from 54 double-helical DNA domains packed on a honeycomb lattice. (d) TEM image showing DNA channels incorporated into a small unilamellar vesicle. (e) Schematics of a CNT porin inserted into a lipid membrane. (f) TEM image showing a CNT porins inserted into a lipid vesicle. Inset shows the relative orientation of the membrane (red) and the CNT porin (blue). Panel c,d: Reproduced with permission.[23] Copyright 2012 by the American Association for the Advancement of Science. Panels e,f: Reproduced with permission.[26] Copyright 2014, Nature Publishing Group.

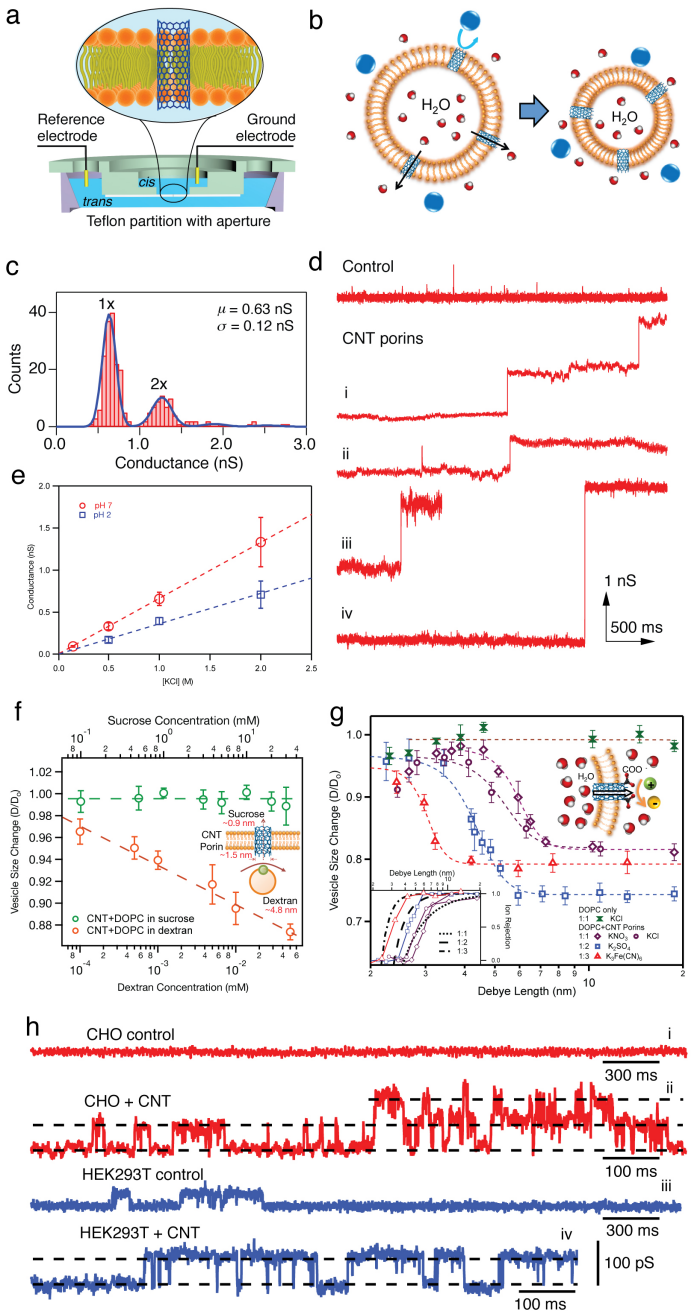

Figure 4. Ion transport in CNT porins in lipid membranes. (a) Schematics of a planar lipid bilayer measurement for detecting single CNT porin incorporation in lipid membranes. Two fluidic chambers are separated by a teflon partition with a small (~200 μm) aperture covered by a lipid bilayer. Two electrodes measure the ion current through the aperture. (b) Schematics of the osmotically-indiced transport measurements through CNT porins. Osmotic gradient between the bulk solution and lipid vesicle lumen space induces water flow out of the vesicle and shrinks the vesicle size. (c) Histogram of the individual CNT porin ionic conductance showing peaks corresponding to incorporation if one (1×), or two (2×) CNT porins. Blue curve represents a fit to a sum of two Gaussian peaks. (d) Conductance traces showing jumps corresponding incorporation of individual CNT porins. Traces *i* and *ii* show individual incorporation events, trace *iii* and *iv* show simultaneous incorporation of two and four CNT porins, respectively. (e). Conductance of CNT porins as a function of the ion

concentration in the solution at two different solution pH values. (f) Plot of the size change of vesicles containing CNT porins after exposure to solution containing different concentrations of sucrose and dextran osmolytes. Dashed lines are guides to the eye. (g) Plot of the vesicle size change as a function of the solution Debye length after exposure to the different 1:1, 1:2, and 1:3 electrolyte solutions of different concentrations. Dotted lines indicate a fit to a sigmoidal function. (Inset) Comparison of the CNT porin ion rejection data (markers and solid lines, same as indicated on the main panel legend) with the predictions of the Donnan model for 1:1, 1:2, and 1:3 electrolyte solution (dashed and dotted lines). (h) Conductance traces demonstrating CNT porin incorporation into the plasma membrane of CHO (ii) and HEK293T (iv) cells. Panels c-e, h: Reproduced with permission.[26] Copyright 2014, Nature Publishing Group. Panels f,g: Reproduced with permission.[27] Copyright 2014, American Chemical Society.

Living systems generate energy and transduce signals by transporting electrons and ions across cellular membranes. To control these biological processes, researchers have created nanostructures that increase the transmembrane flux of electrons and ions. This review summarizes recent advances in the creation these *de novo* and biologically-derived nanostructures and highlights remaining challenges to the widespread use of these materials in biotechnological applications.

Keyword bioelectronics, bioelectrochemical systems, synthetic biology, abiotic-biotic interface

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Title Crossing Over: Nanostructures that Move Electrons and Ions Across Cellular Membranes